A RAT TECHNIQUE FOR DEMONSTRATING THE INTERFERING EFFECT OF CEREALS ON BONE CALCIFICATION

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XVI. A RAT TECHNIQUE FOR DEMONSTRATING THE INTERFERING EFFECT OF CEREALS ON BONE CALCIFICATION.

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E. Mellanby [1922] found that cereals interfere actively with the calcification of bones of dogs and that this action could be completely antagonised by a sufficiency of antirachitic vitamin (vitamin D) in the diet. The intensity of the anticalcifying influence varied greatly with the type of cereal, being greatest in the case of oatmeal and least in the case of white flour, other cereals such as maize, barley, rye, whole meal flour and rice occupying an intermediate position. The experimental results obtained were of greater interest because they were contrary to all previous supposition. The basis for the generally accepted views on the subject was undoubtedly that since oatmeal contained more calcium and phosphorus than, for instance, white flour, it would be expected to be more beneficial to the laying down of these elements in bone. The experiments on dogs completely reversed this expectation. The subject has been continuously followed up by one of us, using puppies as experimental animals, and has formed the basis of publications in which attempts at explaining the phenomena and conditions influencing the action have been described [Mellanby, E., 1924, 1925, 1926]. This work has proved very tedious and slow for various reasons. In the first place, the constituents of cereals are difficult to separate chemically or physically, especially on the scale necessary for the feeding of dogs. The second difficulty is that many of the simple forms of treatment of the cereals destroy the specific toxic effect, and thirdly, only a limited number of experiments, all of a prolonged nature, can be carried out with success at one time.

It was therefore thought advisable to develop a technique which would allow the study of this subject to be pursued on rats. If successful it would obviously simplify matters greatly, and by allowing the use of smaller quantities of prepared material, larger numbers of experiments could be carried out and results obtained more rapidly. It may be remembered that, although experiments on dogs demonstrating the presence of the antirachitic vitamin (now called vitamin D) were first described in 1918 [Mellanby, E., 1918], the general acceptance of this substance as an entity was delayed until other workers obtained confirmatory evidence of its presence by rat experiments in 1921 [McCollum, Simmonds, Shipley and Park, 1921; Korenchevsky, 1921].

It seemed likely that the demonstration of the toxic effects of cereals on rats would not only hasten the acceptance of the facts, but would also attract a larger number of workers to an aspect of nutrition which is probably as important as that of the vitamins, and would thus result in a more rapid identification and isolation of the factor or factors.

The object, therefore, of the present publication is primarily to show that the experimental results on dogs previously published can, in general, be demonstrated with equal ease in rats. No attempt has been made to go beyond this demonstration.

While the present work was being carried out, Dr P. Holst informed us privately that he had been able to demonstrate the interfering effect of cereals on calcification in rats by feeding them entirely on the individual cereals to be tested. It is obvious that such an experimental method must be of limited value, for animals under such conditions lose weight and this must interfere essentially with what is in reality an abnormality of the growth process. In Holst's [1927] results which have now been published the loss of weight of the animals is a prominent feature. Although his results can be regarded as confirming the anticalcifying action of cereals, the loss of weight of the animals prevents them from having a quantitative value. In the technique described below it will be seen that the animals grow rapidly, eat the food well and generally remain in a healthy condition over the required experimental period. Great differences in bone calcification are produced by different cereals, and by the same cereal after different forms of treatment.

Feeding technique.

The results obtained by experiments on dogs made it clear that in order to demonstrate the interfering effect of cereals on the calcification of bones at least two conditions were necessary, (1) that the diet should be deficient in vitamin D and (2) that the diet should not have a high calcium content. In the dog experiments it was shown that a sufficiency of vitamin D completely antagonised even the most powerful cereal effect and allowed the production of perfect bones. The addition of calcium carbonate to diets deficient in antirachitic vitamin improved calcification and tended to convert a rachitic to an osteoporotic condition of the bones. With a diet only slightly deficient in vitamin D, the addition of calcium carbonate often resulted in well calcified bones [Mellanby, E., 1925]. It seemed probable that additional calcium in the diet would be even more potent in antagonising the cereal effect in rats than in dogs, especially if rachitic pathological changes were used as criteria of abnormal calcification. It will be remembered that the development of standard rickets-producing diets in rats by McCollum, Simmonds, Shipley and Park [1921], Korenchevsky [1921] and Sherman and Pappenheimer [1921] necessitated an abnormal calcium-phosphorus ratio as well as a deficiency of the antirachitic vitamin in the diet. In the experimental diets chosen, although the calcium intake was relatively low as

compared with the phosphorus, it must be emphasised that there was no absolute deficiency, for the addition of vitamin D to the diet resulted in the development of well calcified bones without the addition of any more calcium.

In making up the synthetic diet which obviously had to contain a large proportion of cereal or cereal product, the following points were also taken into consideration. (1) The protein intake should be increased above that supplied by the cereal. This was done by adding caseinogen. (2) Substances in which cereals are deficient, namely sodium chloride, vitamin A and vitamin C, should be added. As a source of vitamin A dried cabbage was used, and lemon juice for vitamin C. (3) Since some of the cereal products tested were deficient in vitamins B_1 and B_2, an excess of these should be assured by the addition of marmite.

The basal diets were made up in the following way. A standard mixture was made containing caseinogen, 24 %; sodium chloride, 12 %; marmite, 32 %; lemon juice, 32 %. To 200 g. of this mixture were added 50 g. of dried cabbage. In the earlier experiments only 20 and 25 g. of dried cabbage were added, but in order to ensure good growth and complete freedom from ophthalmia this was ultimately increased to 50 g. 2·5 g. of this mixture was added to 7·5 g. of the cereal or cereal product to be tested and given to each young rat daily. During the early days of the experimental feeding less than this quantity was eaten. The caseinogen was heated at 120° for 36 hours in an electric oven before being added to the diet.

The diet contained only a small amount of vitamin D, dried cabbage being the main source of this, and the amount present was too little to prevent subnormal calcification whatever cereal was added. The same batch of dried cabbage was used in each series of experiments, so that the amount of vitamin D was constant where the results had to be compared. As regards calcium and phosphorus present in the diet, these varied with the type of cereal, both being higher when oatmeal or cereal germ was eaten than when white flour or rice formed the cereal basis. However, the increase in phosphorus was greater than that of calcium, since both in oatmeal and cereal germ the Ca : P ratio was less than that of white flour.

(1) *Duration of feeding experiment.* Young rats of weight between 30 and 40 g. were used. In each series of experiments the duration of feeding was the same but the period varied in different series between 17 and 52 days. The duration of any experiment was decided by a number of factors: (*a*) the importance of getting good differentiation between the calcification in the members of a series; under favourable conditions the longer the experimental period the better is the differentiation produced; (*b*) when, however, a powerful rickets-producing diet is eaten, as for instance when oatmeal forms the cereal basis and there is little or no vitamin D or extra calcium, then, although good growth is obtained in the first part of the experiment, this is often followed at a later period by loss of appetite and weight. Rats respond rapidly to this loss of weight and as in dogs [Mellanby, E., 1918] so in rats the rickety changes

in the bones are decreased and the calcium content increased, thus reducing the value of the experimental results. The experiment should, therefore, be ended, if possible, during the period of growth and not prolonged if the animals begin to lose weight.

(2) *Rate of growth.* Just as it is important to prevent loss of weight of the animal during the experimental period, so is it desirable to maintain, if possible, a fairly equal rate of growth in all the animals in a series. This is difficult, for it is found that after the first few weeks the appetite of the animals eating the diets with white flour as the cereal is much greater than that of the animals whose cereal basis is oatmeal. The amount of food eaten should therefore be regulated with the object of getting equal growth throughout. The increase in weight of the animals in some of the experiments described below was not as even as was desirable. It is hoped that this object will be more nearly obtained in future by closer control of the total intake of food by each rat.

(3) *Calcium content of bones.* E. Mellanby [1921] pointed out that the variations of the fat content of the bone marrow militated against the reliability of interpreting the calcium content of bones in terms of their dried weight. On the other hand, the great variations in size of bones of different animals necessitated due regard to bone weight. In dogs the ratio of calcium content to the original wet weight of a bone is a more reliable index of calcification than the ratio of calcium content to dried bone weight. The error due to fat content is particularly important in cases where the animals are either losing weight or gaining but slowly at the end of the experiment, for under these conditions fat disappears rapidly from the bone marrow. This difficulty has been overcome, in so far as the interpretation of calcification of rats' bones is concerned, by Chick, Korenchevsky and Roscoe [1926], who extracted the fat of the bone before drying and weighing. This method has been adopted in the present experiments. The calcium percentage is given in terms of the weight of the dried fat-extracted bones. The A/R ratio described by Chick, Korenchevsky and Roscoe is also given in the tables below. A represents the ash of the bones and R the difference between the weights of the fat-extracted dried bone and the ash. The bones used for calcium estimation were the femur, tibia and fibula of one leg.

Experimental Results.

I. *The effect of increasing the cereal relatively to the other substances in the diet.*

As in the case of dogs [Mellanby, E., 1920, 1921, 1925], increasing the proportion of cereal eaten and keeping other substances in the diet constant brings about worse calcification of the bones. The following experimental results (Table I) illustrate this fact. In the tables basal diet means the basal substances described above without the added cereal.

Table I.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: A/R ratio
79	31	3·75 g. white flour	F.	44	47	7	18·07	0·95
80	31	,,	M.	50	67	34	19·34	1·09
82	31	7·5 g. white flour	M.	47	74	57	17·61	0·85
83	31	,,	M.	33	70	112	15·48	0·72
84	31	,,	M.	49	94	92	16·39	0·82

It will be seen that doubling the amount of white flour in 82, 83 and 84 reduced the average A/R ratio from 1·02 (79 and 80) to 0·80 (82, 83 and 84). Of course, the increase in cereal brought about increased rate of growth, but that this in itself was not entirely responsible for the more defective calcification is evident from the comparative effect of different cereals described below. As has already been pointed out in puppies [Mellanby, E., 1922, 1925], although equal growth may be produced by different cereals, there is great variation in calcification.

II. *The effect of different cereals on calcification.*

The method described above of feeding rats also allows great differences in the anticalcifying effect of different cereals to be demonstrated. The results are, on the whole, in agreement with those previously obtained by experiments on dogs. Some examples of experimental results are given in Table II.

Table II.

Series 1.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: A/R ratio
35	28	White flour	M.	37	106	186	17·41	0·85
37	28	Oatmeal	M.	33	66	100	14·08	0·61
39	28	Wholemeal flour	M.	40	95	138	15·83	0·75
45	28	Maize meal	M.	34	77	126	17·10	0·86
47	28	Barley meal	M.	31	90	180	18·31	0·98
49	28	Rice	M.	31	73	135	16·67	0·82

Series 2.

36	42	White flour	F.	30	95	217	18·85	1·04
38	42	Oatmeal	F.	35	84	140	15·34	0·70
40	42	Wholemeal flour	F.	31	82	164	14·94	0·67
46	42	Maize meal	F.	31	72	132	16·24	0·78
48	42	Barley meal	F.	29	96	265	19·60	1·13
50	42	Rice	F.	32	68	112	15·22	0·70

It will be noticed that the first series lasted 28 days and the second series 42 days.

There is fair agreement in the results of the two series. Oatmeal and wholemeal flour have brought about the worst calcification of the bones, while barley meal and white flour are associated with the best calcified bones.

The first of the following series (Table III) also emphasises the relatively potent anticalcifying action of oatmeal as compared with white flour.

Table III.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: *A/R* ratio
56	42	White flour	F.	35	81	131	15·60	0·73
57	42	,,	F.	33	81	145	15·93	0·75
58	42	Oatmeal	F.	34	77	126	13·11	0·63
59	42	,,	M.	33	68	106	13·04	0·59
117	30	White flour	M.	37	65	76	16·94	0·81
120	30	Rice	M.	52	57	10	16·23	0·78
122	30	,,	M.	38	51	34	16·11	0·77

The last two experiments (120 and 122) in which rice was the cereal eaten are given to illustrate one of the difficulties met with in these experiments. It is the most obvious instance in which this technique at present fails because in the majority of cases in which rice is used the growth obtained is poor and so does not allow a good comparison with other cereals to be made. Why the growth should be so relatively poor in the rice experiments is not known. If starch be used instead of a cereal, very poor growth also results, as is seen in the next experiments (Table IV).

Table IV.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: *A/R* ratio
1	52	White flour	M.	49	113	131	17·24	0·89
6	52	Starch	F.	39	46	18	17·02	0·91
7	52	,,	F.	30	50	67	17·06	0·94
12	52	Oatmeal	F.	29	60	107	13·06	0·60

The low calcium content of the bones of Exps. 56 and 57 of Table III, where the animals ate white flour as the cereal basis, requires comment. The probable reason for these results as compared with those obtained in animals eating similar diets and recorded in other tables is that the vitamin D content of the cabbage eaten was smaller than usual. Since all the animals of the series received the same cabbage, the individual results are comparable with each other although not with those of other series.

The results obtained with starch not only show that, under these dietetic conditions, the growth of the animals is poor, but suggest that the carbohydrate moiety of the cereal is not responsible for the anticalcifying effect. This is confirmatory of the results on dogs [Mellanby, E., 1922, 1925].

In the dog experiments previously referred to [Mellanby, E., 1925] it was found that wheat germ also interfered with bone calcification. This fact can also be seen in rat experiments (Table V).

Table V.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: *A/R* ratio
119	24	White flour	M.	39	75	90	17·37	0·89
124	24	73 % white flour and 27 % wheat germ	M.	32	68	119	14·94	0·67
127	24	73 % white flour and 27 % maize germ	F.	32	52	62	15·95	0·76
118	30	White flour	M.	57	100	75	17·76	0·92
123	30	73 % white flour and 27 % wheat germ	M.	38	74	94	12·76	0·52
126	30	73 % white flour and 27 % maize germ	F.	28	53	89	15·32	0·71
128	30	73 % white flour and 27 % maize germ	M.	34	47	38	15·41	0·71

In these experiments 27 % of either wheat or maize germs was substituted for 27 % of the white flour. It is evident that both types of germ interfere with bone calcification.

III. *Influence of salts containing calcium and phosphorus on the cereal effect.*

In the dog experiments it was found that the effect of the cereals could be modified by altering the calcium and phosphorus of the diet [Mellanby, E., 1925]. The addition, for instance, of calcium carbonate, especially to a diet containing oatmeal as cereal, tended to improve calcification and convert rachitic changes of bones into osteoporosis. The improvement in calcification was much less than that produced by adding sources of antirachitic vitamin. Calcium phosphate, in the absence of vitamin D, produced a smaller improvement than calcium carbonate. The importance of these observations was that they affected the question as to the cause of the cereal effect. One of the most obvious suggestions as to causation was that the anticalcifying influence of cereals was due to their calcium-phosphorus ratio, for both oatmeal and wheat germ have a relatively low calcium-phosphorus ratio as compared with white flour. Since, also, raising the ratio by adding calcium carbonate decreased the rickets-producing effect, the hypothesis that the action of cereals could be explained on the basis of this ratio was strengthened. The problem of the part played by the calcium-phosphorus balance in the aetiology of rickets and bone calcification has loomed large in experimental work since its importance in experiments on rats was pointed out by McCollum, Simmonds, Shipley and Park [1921]. The whole question from the point of view of cereals was considered by one of us and the conclusion was reached that the balance of evidence did not yet justify the belief that this simple explanation covered all the facts [Mellanby, E., 1925]. This point, however, is still not settled. The following experiments with rats (Table VI) show that the addition of calcium salts antagonises the anticalcifying effect of cereals in the absence or great deficiency of vitamin D. They differ from the results obtained

with dogs in that calcium phosphate seems to bring about as great an improvement in calcification as calcium carbonate. It may be remembered that in the dog experiments this only happened when a source of fat-soluble vitamin such as butter was included in the diet.

Table VI.

No. of exp.	Duration in days	Cereal and salt added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: A/R ratio
		Series 1.						
94	17	Oatmeal	F.	35	77	120	16·49	0·78
96	17	,,	M.	40	85	111	16·11	0·77
102	17	Oatmeal + 0·5 % $CaCO_3$	M.	40	81	103	18·05	0·95
103	17	,,	F.	52	106	104	18·91	1·04
107	17	Oatmeal + 0·5 % $Ca_3(PO_4)_2$	F.	38	76	100	17·81	0·92
108	17	,,	M.	44	86	95	17·20	0·87
109	17	Oatmeal + 0·5 % K_3PO_4	M.	42	73	74	15·57	0·72
112	17	,,	F.	55	90	64	17·10	0·86
		Series 2.						
93	24	Oatmeal	F.	30	66	120	16·48	0·80
101	24	Oatmeal + 0·5 % $CaCO_3$	M.	35	78	123	17·77	0·92
105	24	Oatmeal + 0·5 % $Ca_3(PO_4)_2$	M.	32	68	113	17·81	0·93
110	24	Oatmeal + 0·5 % K_3PO_4	M.	25	57	128	17·24	0·87
		Series 3.						
95	30	Oatmeal	M.	42	94	124	15·41	0·71
104	30	Oatmeal + 0·5 % $CaCO_3$	M.	38	93	145	18·03	0·95
106	30	Oatmeal + 0·5 % $Ca_3(PO_4)_2$	F.	48	100	108	19·50	1·11
111	30	Oatmeal + 0·5 % K_3PO_4	F.	35	63	80	15·63	0·73

These experimental results show that the addition of either calcium carbonate or calcium phosphate to the diet improves calcification and that there is little or no difference between the action of the two salts. That it is the calcium which is important is also evident from the fact that potassium phosphate had much less effect on the calcification of the bone. The effect of potassium phosphate in these experiments was somewhat variable; in two of the above cases there was slight improvement, in one slight impairment of calcification and in one case no effect. At all times the effect was small. It would appear that the addition of calcium is of more importance in itself than because of any influence the added salts have on the calcium-phosphorus ratio. If this ratio were crucial it would be expected that the addition of potassium phosphate would have made bone calcification consistently worse, but this is not the case.

The figures in Table VII represent the approximate calcium and phosphorus amounts and the calcium-phosphorus ratios of the above diets.

Table VII.

	Addition to basal diet	Ca %	P %	Ca/P ratio	Effect on bone calcification
1.	Oatmeal	0·205	0·47	0·42	Very bad
2.	Oatmeal and $CaCO_3$	0·35	0·47	0·74	Much better than 1
3.	Oatmeal and $Ca_3(PO_4)_2$	0·32	0·58	0·55	Like 2 and much better than 1
4.	Oatmeal and K_3PO_4	0·20	0·58	0·34	Similar to 1

IV. *The antagonism of the cereal effect by vitamin D.*

Even the most potent anticalcifying effect of cereal, such as that produced by oatmeal, can be overcome by a sufficiency of the antirachitic vitamin in dogs [Mellanby, E., 1925]. The following experiments (Table VIII) show the effect of adding 2·5 and 5 mg. of cod-liver oil daily to the basal diets containing oatmeal.

Table VIII.

No. of exp.	Duration in days	Cereal and oil added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	*A/R* ratio
			Series 1.					
88	25	Oatmeal	F.	50	72	44	16·25	0·78
89	25	Oatmeal + 2·5 mg. cod-liver oil	M.	45	83	84	17·80	0·96
			Series 2.					
94	17	Oatmeal	F.	35	77	120	16·49	0·78
96	17	,,	M.	40	85	111	16·11	0·77
98	17	Oatmeal + 5 mg. cod-liver oil	M.	34	69	103	18·08	0·96
99	17	,,	M.	35	73	109	18·15	0·96
			Series 3.					
93	24	Oatmeal	F.	30	66	120	16·48	0·80
100	24	Oatmeal + 5 mg. cod-liver oil	F.	38	99	161	19·71	1·14
			Series 4.					
95	30	Oatmeal	M.	42	94	124	15·41	0·71
97	30	Oatmeal + 5 mg. cod-liver oil	M.	36	119	231	19·38	1·10

These figures show that even 2·5 mg. of cod-liver oil bring about some improvement in calcification, while 5 mg. result in great improvement.

V. *The irradiation of oatmeal by means of a mercury vapour lamp.*

The diminution of the rickets-producing effect by irradiating cereals as shown in dogs [Mellanby, E., 1925], can also be demonstrated in rats by the above technique. In the experiments in Table IX fine oatmeal was exposed to the

rays of a mercury vapour lamp for 30 minutes at a distance of a foot. The oatmeal was scattered on plates and stirred frequently during the time of exposure.

Table IX.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: A/R ratio
66	28	Oatmeal	M.	44	97	143	15·78	0·66
77	28	Irradiated oatmeal	M.	40	102	155	17·38	0·88
64	35	Oatmeal	M.	42	80	90	13·45	0·57
65	35	,,	M.	31	72	132	14·45	0·63
76	35	Irradiated oatmeal	M.	32	97	203	17·58	0·90
78	35	,,	M.	30	95	216	18·93	1·05

In view of recent work, especially that of Rosenheim and Webster [1927] and of Hess and Windaus [1927], there can be little doubt that the improvement in calcification of bones brought about by irradiating oatmeal is due to the formation of vitamin D (antirachitic vitamin) from its parent non-active substance, ergosterol, in the oatmeal.

VI. *The destruction of the anticalcifying action of cereals by acids.*

It has been shown above that vitamin D (antirachitic vitamin), whether given in foods containing it or produced in the food by exposure to a source of ultra-violet radiation, antagonises the anticalcifying action of cereals. In the experiments now to be described the rickets-producing effect of cereals was reduced not by the addition of an antagonising substance but by the actual destruction of the offending agent in the cereal by acid. The cereal was first boiled with 1 % hydrochloric acid until the mixture gave no colour with iodine, *i.e.* until the starch was hydrolysed. The mixture was then neutralised (p_H 6) and added to the diets so as to correspond with the actual amount of cereal given to the control animals. In the control experiments, *i.e.* where oatmeal itself was eaten, an amount of NaCl was added equivalent to that contained in the diets in which the oatmeal had been boiled with hydrochloric acid and neutralised (Table X).

Table X.

No. of exp.	Duration in days	Cereal added to basal diet	Sex	Init. weight g.	Max. weight g.	% increase weight	Ca in bones: % of dry weight after fat extraction	Ca in bones: A/R ratio
58	42	Oatmeal	F.	34	77	126	13·11	0·63
59	42	,,	M.	33	68	106	13·04	0·59
60	42	,,	F.	37	53	72	15·02	0·69
61	42	Acid oatmeal	M.	35	68	94	17·86	0·93
62	42	,,	F.	35	75	114	17·87	0·93
63	42	,,	M.	36	80	122	18·32	1·00

It is clear from these results that boiling oatmeal with 1 % acid in this way destroys to a great extent its power to interfere with bone calcification. It is important to note that the time of boiling and the strength of the acid

used are of great significance. Holst [1927] has indeed found that the rickets-producing effect of oatmeal can be obtained in a filtrate obtained by boiling this cereal with 0·5 % hydrochloric acid. Thus, whereas a short period of boiling with dilute acid brings the rickets-producing substances into solution, more prolonged boiling of the cereal destroys this substance. This part of the subject will be dealt with more fully in a subsequent paper.

Conclusions.

The experimental work described above shows that it is possible by suitable dietetic methods to demonstrate in rats the anticalcifying actions of cereals similar to those previously demonstrated by one of us in dogs [Mellanby, E., 1922, 1925, 1926]. The feeding technique, although undoubtedly capable of improvement, allows good growth and, except in the worst rickets-producing diets, fair health during the experimental period. It has been found possible (*a*) to make the intensity of rickets worse by increasing the cereal intake when the other dietetic ingredients are kept constant; (*b*) to demonstrate great differences in the rickets-producing effects of different cereals, oatmeal having the most potent and white flour the least action in this respect; (*c*) to demonstrate the rickets-producing effect of germ, both of wheat and maize; (*d*) to show the antagonism of substances containing vitamin D to the cereal action or to lessen the effect by exposing the cereal to ultra-violet radiations; (*e*) to show that raising the calcium of the diet either by adding calcium carbonate or calcium phosphate minimises the anti-calcifying action of cereals; and (*f*) to demonstrate the ultimate destruction of the anticalcifying action of cereals by boiling with dilute hydrochloric acid.

What constituent of the cereal is responsible for this powerful property of interfering with bone calcification has not been discussed in this paper, but the simplified technique described ought to allow a more rapid accumulation of facts concerning this important side of dietetics.

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